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ORIGINAL RESEARCH PAPER

Assessment of *Salix* spp. pollen availability to insects based on aerobiological investigations

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Abstract
Pollen and nectar produced by flowers of species from the genus *Salix* are an important source of food for various insect groups in early spring. Most willows are entomophilous species; however, substantial amounts of airborne *Salix* pollen can be noted. The aim of the study was to evaluate the content of pollen of this taxon in the air of Lublin (central-east Poland) in 2001–2016 and to identify the period of its greatest availability to insects. In 2015, we compared the course of the *Salix* pollen season in Lublin (51°14′37″ N; 22°32′25″ E) and in the Roztoczański National Park (50°34′57″ N; 23°04′24″ E), Poland. We found that the date of the pollen season onset fluctuated greatly between March 16 and April 17. The greatest availability of *Salix* pollen to insects was noted from the end of the first 10-day-period of April to the first 10-day-period of May. The mean annual sum of airborne *Salix* pollen grains was 833. In Lublin, *Salix* pollen accounted for ca. 1.25% of the total airborne pollen content of different plant taxa. The investigations have demonstrated a 2-year cycle of *Salix* pollen abundance. The comparison of the pollen seasons in Lublin and in the Roztoczański National Park indicates that considerably greater amounts of pollen occur in the urban area than in the air of the Roztoczański National Park.

Keywords
bioaerosol; pollen seasons; dynamics; annual sum; Lublin; *Salix* spp.

Introduction

The genus *Salix* (Salicaceae) comprises over 400 species [1]. In Europe, there are ca. 65–70 representatives of the genus [2–4]. Willows include many interspecific hybrids [5]. *Salix* species are woody, heliophilous, pioneer plants, which rapidly colonize new habitats. They usually grow in river valleys and wetlands, creating riparian forests. Wild willow species are common worldwide from the Arctic to the tropical zone. Some of them grow on stony substrates in the mountains at an altitude over 2500 m [6,7]. In Poland, willows grow in forests, along riverbanks, and in wet meadows across the country. They are used in landscape plantings, in parks, and along roads and streets. Willows are tall trees, shrubs, and dwarf shrublets growing in the mountains or in the far north [1,8]. In Poland, *S. cinerea*, *S. fragilis*, *S. purpurea*, and *S. viminalis* are the

most common [5,9]. The *S. purpurea* and *S. viminalis* are widespread in Europe and used for stabilization of riverbanks, dikes, and dunes and for furniture production and basketry [4,10]. Lately, *S. viminalis* has been cultivated on a large scale as an energetic plant due to its high biomass production [11,12].

Willows are dioecious plants. The flowers are arranged in catkin inflorescences [6,7]. From the point of view of pollination ecology, *Salix* inflorescences represent brush-like flowers [13]. Perianth is absent in both male and female flowers. Male inflorescences are more prominent due to their intense yellow color of the stamens [6]. Most willow species are entomophilous plants [14] offering pollinators abundantly produced nectar and pollen rewards. *Salix* flowers can be pollinated by representatives of Coleoptera, Diptera, Hymenoptera, Lepidoptera [13], and Apoidea (particularly by *Apis mellifera*, *Bombus* spp.) [11,14]. Some species, notably alpine and arctic, are anemophilous [1]. In Norway, *Salix* species are pollinated both by wind and insects [15]. The authors found that the anemophily varied between 2% and 52%. The female *Salix* flower is composed of a small leaflet called a bract, a yellow-green pistil, and 1–2 nectaries. The male flower has 2–12 stamens, a bract, and 1–2 nectaries [6,13]. The nectaries in both types of flowers secrete nectar, which contains 12–20% of sugars in European species and ca. 60% of sugars in American species. Unifloral honeys from willow species growing in the Netherlands, Finland, and Germany are known [6]. However, most frequently, the willow nectar is a component of multifloral honeys [6,16,17].

Salix pollen contains 4% of nitrogen and 15–22% of proteins and is recognized as highly valuable to bees [6,14]. Although willow flowers are mainly entomophilous, the pollen is transported by wind as well, which is related to the absence of the perianth and the good exposure of stamens – typical features of anemogamous plants [18]. Aerobiological investigations revealed presence of high contents of airborne *Salix* pollen in some years [19]. However, in contrast to the pollen of many anemophilous tree taxa, *Salix* pollen is thought to exert a low allergenic effect [20].

Results of aerobiological investigations of the content of willow pollen grains in the air facilitate determination of the period of abundant pollen release of *Salix* available to insects. The period of maximum *Salix* pollen concentration is likely to coincide with the full bloom of several *Salix* species and is associated with intensive nectar secretion. The high concentrations of *Salix* pollen may indicate the presence of many representatives of willow species in the landscape. The March–May period is the time of flowering of most *Salix* species in Poland [8,14].

The aim of the present study was to identify the onset and end of the *Salix* pollen season, determine the abundant pollen release period, and estimate the maximum concentrations and annual pollen sums based on 16-year aerobiological studies conducted in the urban area of Lublin. Since the global warming noted over the recent decades has altered plant flowering and the amounts of produced pollen, trend lines for annual pollen sums, maximum concentrations of pollen grains, and the onsets of the *Salix* pollen seasons were established. In 2015, the *Salix* pollen data form Lublin have been compared with pollen data from Guciów, a village in the Roztoczański National Park (located 100 km away from Lublin). In the paper, the content of airborne *Salix* pollen in various regions of Poland was also compared with relevant data from some European regions

Material and methods

In Lublin, the flora of parks and squares located along the Bystrzyca River, and the flora of fragmented forests on the outskirts of the city provide habitats for the following *Salix* species: *S. fragilis* L. (Fig. 1a,b) (April–May), *S. alba* L. (April–May), *S. alba* ssp. *vitellina* (L.) Arcang. (April–May), *S. aurita* L. (March–May), *S. caprea* L. (Fig. 1c,d) (March–April), *S. triandra* L. (April–May), and *S. viminalis* L. (March–April) [21,22]. In the Roztoczański National Park, near Guciów: *S. aurita* L. (March–May), *S. caprea* L. (March–April), *S. cinerea* L. (March–April), and *S. fragilis* L. (April–May) were grown [21,23]. Periods of blooming are given in brackets. The dates of flowering follow those identified by Rutkowski [8]. We measured anthers of *S. caprea* ($N = 20$) and stained its pollen grains with toluidine blue and Sudan IV.

Measurements of the *Salix* pollen concentration were performed in Lublin (51°14′37″ N; 22°32′25″ E, 197 m a.s.l.) central-east Poland in 2001–2016 (Fig. 2). Aeropalynological analyses were carried out using the volumetric method with a Hirst-type device (Lanzoni VPPS 2000). The pollen trap was located near the city center on a flat roof of a building at a height of 18 m above the ground.

The research was conducted in accordance with standard aerobiological methods recommended by the International Association for Aerobiology [24]. In 2015, the measurements of the pollen grain concentration were also performed in Guciów, a village located 100 km south-east of Lublin (L). The pollen trap was located on a flattened left slope of the Wieprz River valley (50°34′57″ N; 23°04′24″ E, 255 m a.s.l.) in a meteorological plot of the Roztocze Research Station (RNP), Maria Curie-Skłodowska University at a height of 3 m above the ground. The results were expressed as the number of pollen grains per 1 m^3 of air per day (P/m^3).

The dates of the pollen season were determined using the 98% method [25,26]. The study consisted in analysis of the course of the pollen seasons in each year, the dates of the onset of the seasons, the maximum pollen concentrations, and annual pollen sums. A linear trend was determined for some of the pollen season characteristics. Spearman's correlation between the parameters of the *Salix* pollen season and between the *Salix* pollen season parameters and meteorological factors, i.e., minimum, mean, and maximum temperature, humidity and wind speed, were calculated.

Results

During the aerobiological study, we focused our attention on insects visiting willow flowers. Pollen and nectar foraging by bumblebees (Fig. 1c) and bees (Fig. 1e) was observed on *S. caprea* stamen inflorescences. The anthers length was 1.26 mm, on average. Pollen grains are tri-zonocolpate with pollenkitt on the surface (Fig. 1f–h).

The beginning of flowering of the *Salix* species and the onset of the *Salix* pollen season in Lublin were noted between March 6 and April 17 during the 16-year study period. The substantial difference (up to 41 days) was related to the high temperature fluctuation in March/April. The trend line determined for the dates of the pollen season onset suggests a slight, statistically insignificant downward trend indicating somewhat earlier development of male *Salix* flowers over the successive study years (Fig. 3).

The graphs showing the dynamics of the *Salix* pollen seasons reveal many peaks in the curves, which differ significantly in their height and occurrence dates over the consecutive years (Fig. 4). The presence of many peaks during the pollen season may indicate successive flowering and pollen release by different willow species. In 2001–2016, maximum concentrations of *Salix* pollen grains were recorded between April 11 and May 1. The *Salix* pollen concentrations ranged 25–223 pollen grains per 1 m^3/day (Fig. 5), with a mean of 115 P/m^3/day. During the study years, high *Salix* pollen concentrations (over 30 P/m^3) were recorded between the end of the first decade of April and the beginning of the first decade of May (Fig. 6). The course of the trend line for the maximum *Salix* pollen concentrations in Lublin over the 16 study years indicated a decline in the values of the pollen concentrations (Fig. 5).

The annual sums of *Salix* pollen grains ranged between 280 and 1277 grains, with the mean value of 833. The trend line indicates the presence of decreasing values of the sums over the recent years (Fig. 7). The percentage of *Salix* pollen in the sum of all pollen grains deposited in the individual study years in Lublin was in the range of 0.9–2.1% (mean 1.25%). The comparison of the annual sums from the consecutive years indicates alternations in the abundance of pollen release, although the period 2007–2008 was an exception to the rule (Fig. 7).

The significant correlation coefficients between the parameters of the *Salix* pollen season are presented in Tab. 1. The analysis of the relationships between the season parameters revealed the highest correlation between the date of the onset and the length of the pollen season. A season that began later lasted a shorter time (negative correlation). The calculations also demonstrated that the season peak was noted earlier after an earlier onset of the season (positive correlation) and at its greater length (negative correlation). The annual total values were higher in a season that ended earlier, as

Fig. 1 Fragment of a tree and inflorescences of two *Salix* species. **a** General habit of the *Salix fragilis* crown. **b** *Salix fragilis* sprig with male catkins. **c** *Bombus* sp. on a *S. caprea* male inflorescence. **d** *Salix caprea* male inflorescences with numerous stamens. **e** *Apis mellifera* with pollen loads on *S. caprea* male inflorescence. **f–h** Pollen grains of *S. caprea* (**f** – stained with toluidine blue; **g** – stained with Sudan IV; **h** – without staining); arrows show pollenkitt.

Fig. 2 The location of study area and the *Salix* pollen traps in urban area of Lublin (51°14′37″ N; 22°32′25″ E) and in the Roztoczański National Park (50°34′57″ N; 23°04′24″ E), Poland.

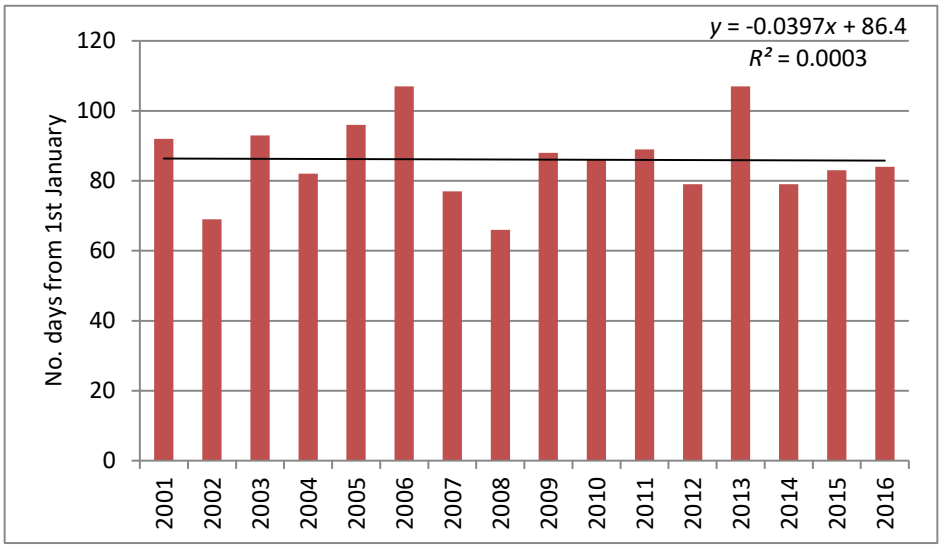

Fig. 3 Trend line and histogram of the dates of *Salix* pollen season onsets in Lublin in 2001–2016.

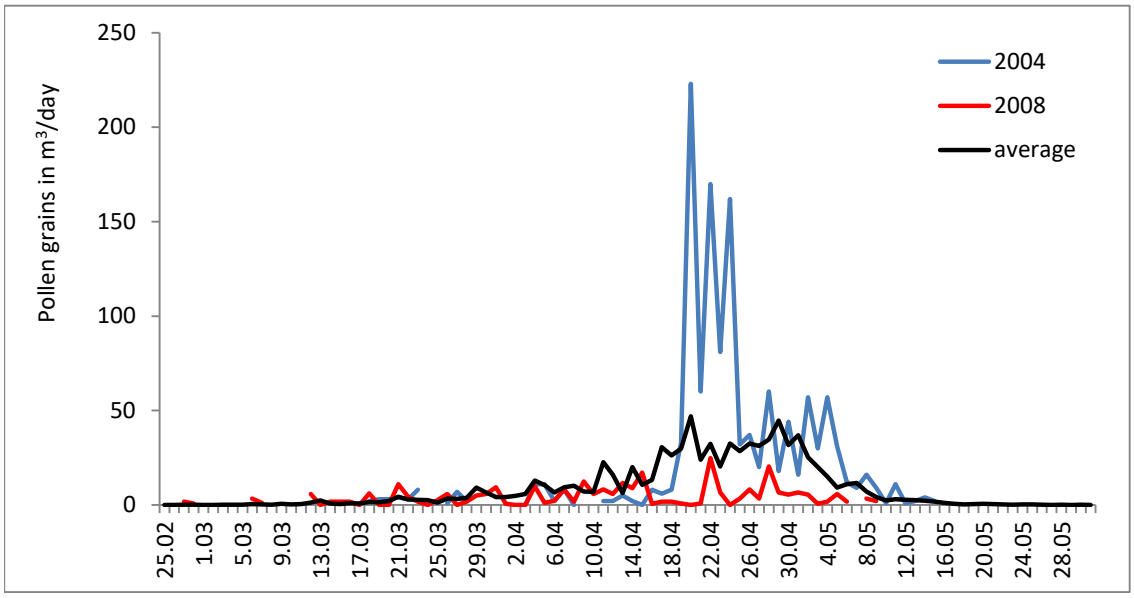

Fig. 4 Daily *Salix* pollen concentrations in the years with the highest (2004) and lowest (2008) annual total value of pollen grains against average value from 2001–2016.

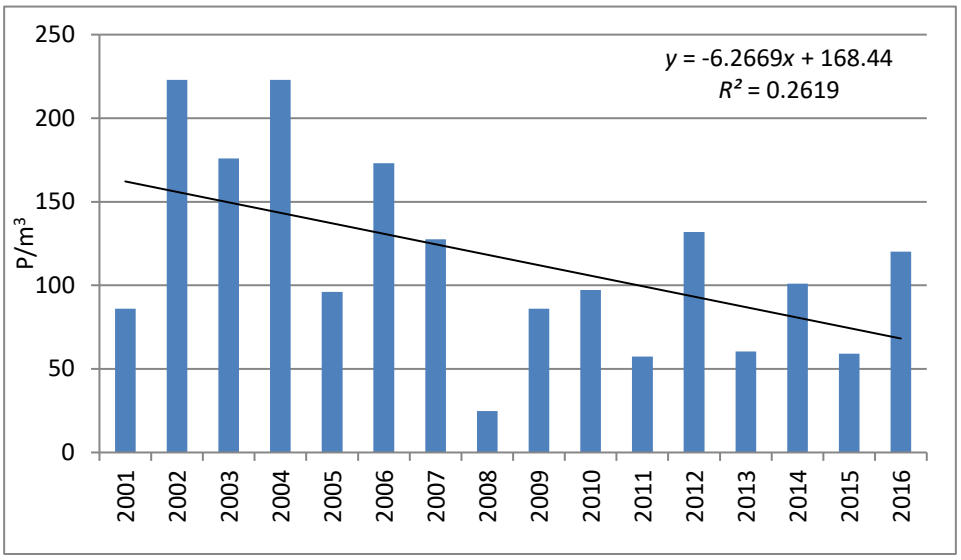

Fig. 5 Comparison of the maximum concentrations of airborne *Salix* pollen grains in Lublin in 2001–2016 with regard to the trend line.

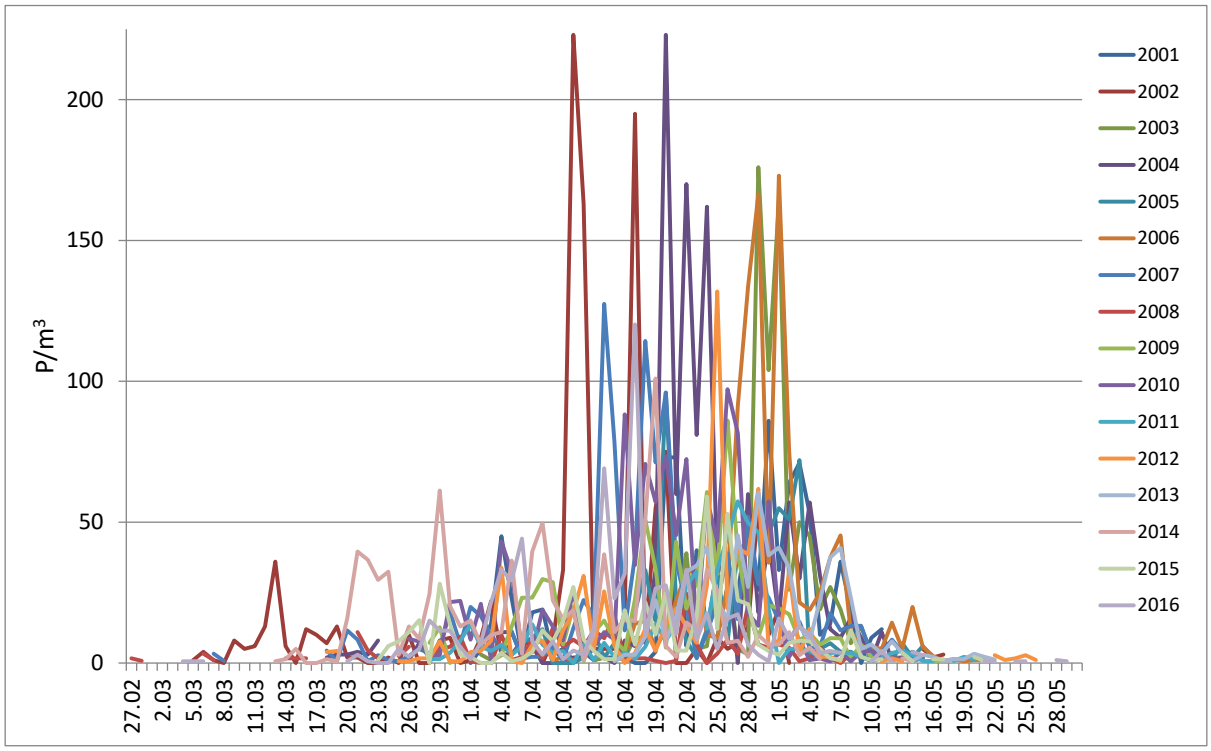

Fig. 6 Comparison of the course of *Salix* pollen seasons in Lublin in 2001–2016.

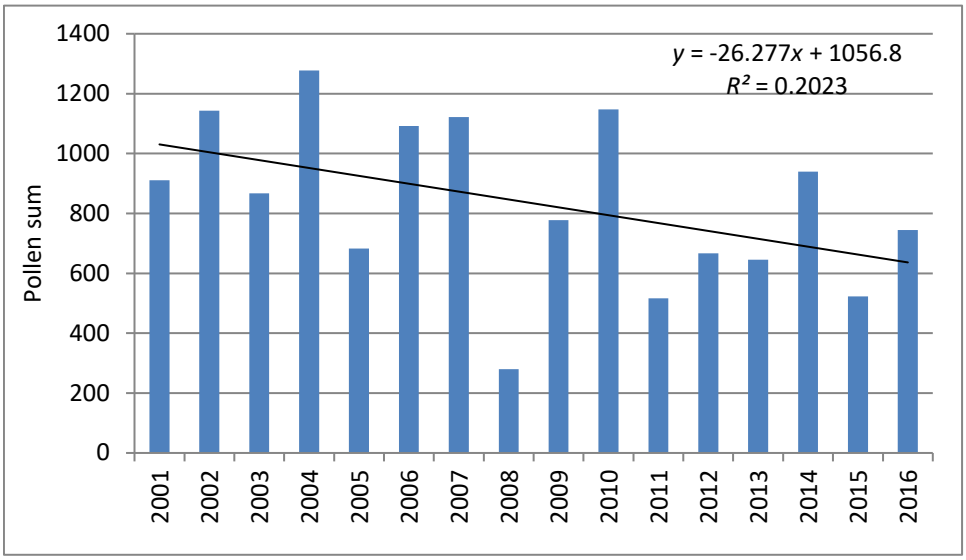

Fig. 7 Comparison of annual sums of *Salix* pollen grains in the air of Lublin in 2001–2016 with regard to the trend line.

Tab. 1 The list of significant Spearman's correlations between the parameters of the *Salix* pollen season in Lublin (2001–2016).

Parameters of pollen season	Spearman coefficient
Start and Duration	−0.8791
Start and Peak Date	0.7432
Duration and Peak Date	−0.6590
End and Annual Total	−0.6232
Peak Value and Annual Total	0.7447

Level of significance 0.01.

indicated by the negative correlation. A positive correlation was found between the peak value and annual total. The results of the statistical analyses of the parameters of the *Salix* pollen seasons and meteorological factors are shown in Tab. 2. The values of Spearman's correlation coefficients indicate that the season onset is significantly influenced by the mean and maximum temperatures in February. In turn, the temperature in January and March and the humidity value in February exert an effect on the length of the season. The peak value depends on the minimum temperature in January and the peak date depends on the temperature in February and March. The annual total value is significantly influenced by the minimum temperature in January.

The comparison of courses of the *Salix* pollen seasons in Lublin (L) and the Roztocze Research Station (RNP) was shown in Fig. 8. Substantial differences were noted in the dynamics of the *Salix* seasons. The disparities in the periods of maximum pollen concentrations (L April 24, RPN March 30) and pollen values (L 59 P/m^3, RNP 21 P/m^3) were noted. The annual sum of *Salix* pollen grains in Lublin was 523, whereas this value in the RNP was several-fold lower, i.e., only 74.

Discussion

During the 16-year study, we showed that in Lublin, i.e., a city located in central-east Poland, the *Salix* pollen season indicating flowering of male flowers in various willow species lasts on average from March 20 to May 10. The main part of the *Salix* pollen season characterized by high content of airborne pollen (over 30 P/m^3) was noted between April 7 and May 7. For honeybee colonies, this is a very important period of development with high demand for nutrition. Not many nectar and pollen yielding plant species bloom during this period [14,27]. The investigations conducted by Koter [28] indicate that many *Salix* species produce considerable amounts of pollen and nectar. For example, *Salix daphnoides*, *S. erdingeri*, and *S. caprea* produce the greatest quantities of pollen per inflorescence: 34.7 mg, 32.6 mg, 31.2 mg respectively. Therefore, *Salix* species provide bees with very important early spring reward [6,14,27]

Tab. 2 Significant Spearman's correlations between *Salix* pollen season parameters and meteorological factors in Lublin in years 2001–2015.

Dependent variable: Season Start	
Mean temperature in February	−0.5206*
Maximum temperature in February	−0.5760*
Dependent variable: Duration	
Mean temperature in January	0.5362*
Mean temperature in March	0.7310**
Minimum temperature in March	0.7542**
Maximum temperature in March	0.6524**
Humidity in February	−0.5451*
Dependent variable: Peak Value	
Minimum temperature in January	0.5331*
Dependent variable: Peak Date	
Mean temperature in February	−0.5228*
Mean temperature in March	−0.7090**
Minimum temperature in March	−0.7270**
Maximum temperature in February	−0.5318*
Maximum temperature in March	−0.6517**
Dependent variable: Annual Total	
Minimum temperature in January	0.6077*

Level of significance: *0.05; **0.01.

Due to the absence of the perianth, the pollen and nectar in *Salix* flowers is continuously available to insects. Bees are highly active while collecting the reward. The *Salix* pollen collected by these insects forms large, light or dark yellow pollen loads, which are sometimes olive brown. The pollen loads are often "interwoven" with hairs bitten off from catkin inflorescences [6].

Honeys produced from willow nectar are similar to fruit tree honeys. They are bright yellow and have a mild flavor and delicate aroma [6]. The quality of honey is assessed based on the content of pollen from various taxa [29]. Willow pollen has been detected in vast amounts in honeys originating from different regions of Poland and other countries. In the study conducted by Warakomska [30] in Poland, *Salix* pollen was found in 80% of analyzed honey samples from Lubelszczyzna. Wróblewska [31] demonstrated that many honeys originating from Podlasie exhibit characteristics of willow species honeys with up to 84% *Salix* pollen content. Multifloral honeys most often contain 16–45% of secondary willow pollen. In 80% of analyzed multifloral honeys from Świętokrzyskie Province, the *Salix* pollen content varied and was present in amounts indicating that it could be considered as secondary pollen [16,32]. In honeys from the Sandomierska Upland area, the content of *Salix* pollen was lower and it was referred to "important minor" (3–16%) or "minor pollen" (<3%) [17]. Unifloral honeys produced from *Salix* nectar have been found in various European countries [33]. A large percentage of *Salix* pollen, i.e., 79% in May and 13% in June, was reported in honeys produced in the northern region of Lithuania [34].

Determination of the annual sums of airborne *Salix* pollen grains facilitates assessment of the abundance of pollen released during different seasons. Based on the multiyear study, periodicity of abundant and poor pollen release by some trees can be shown, which may help to assess the abundance of willow reward. The pollen release rhythm

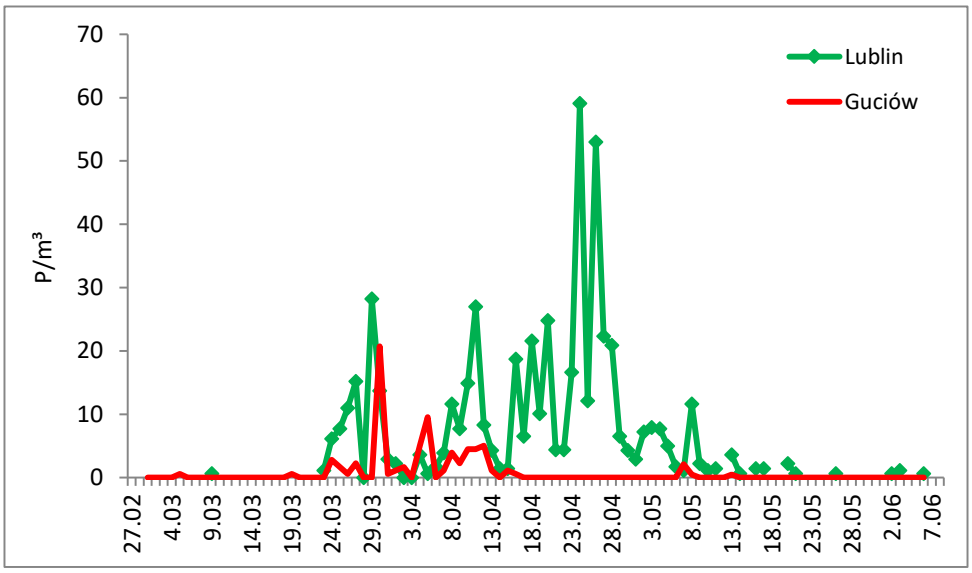

Fig. 8 The course of *Salix* pollen seasons in Lublin and Guciów in 2015.

has been specified for birch [35] and alder [36]. The curves of the dynamics of *Salix* pollen release in the individual years and the annual totals observed in our study allow a conclusion that 2001–2006 and 2009–2016 were characterized by a biennial cycle of pollen abundance. In turn, such regularity was hardly noticeable in 2007–2008.

The annual sums of *Salix* pollen grains in Lublin over the 16 years of the study ranged from 280 to 1277, which is a high value in the case of an entomophilous plant. This may imply high pollen production by willows. Given the small size of the pollen grains in some *Salix* species that are most common in Poland, i.e., from 17.7 µm to 28.0 µm [37], it can be assumed that the relatively large *Salix* anthers in *Salix caprea* (0.97–1.43 mm) can produce great amounts of pollen. Importantly, despite the presence of abundant pollenkitt on the surface of *Salix* pollen, which sticks grains together and hinders the release of single grains from the anthers, the air contains substantial quantities of the pollen of this taxon.

A substantially lower amount of *Salix* pollen was found in the aeroplankton of the Roztoczański National Park (7-fold lower) than in Lublin, which is probably associated with the presence of a greater number of *Salix* species on the outskirts of the city and in the urban greenery. This may also explain the much earlier occurrence of the maximum pollen concentration in RNP than in Lublin; it was noted at the end of March, which was related to the earlier onset of flowering of several *Salix* species (*S. caprea*, *S. cinerea*, *S. aurita*) growing in this area [21,23].

The comparison of the mean annual sums noted in some cities of Poland in 2001–2005 demonstrated that the greatest amount of *Salix* pollen was recorded in Lublin, where the annual sum was 976 [19], and in Rzeszów and Szczecin with the mean values of 783 and 732 grains, respectively [38,39]. The lowest pollen sums were noted in Wrocław, Łódź, and Poznań, i.e., 104, 322, and 323, respectively [40–42].

In the present study, we found that *Salix* pollen accounted for 1.25%, on average, of the total pollen content in the aeroplankton of Lublin during the growing season. Pollen calendars from different regions of Europe indicate that *Salix* pollen represents varied percentage participation. For instance, it was estimated at 1.12% in Germany (Münster) [43], 1.36% in Slovakia (Bratislava) [44], and 1.10% in Sweden (Stockholm) [45]. These values are similar to those recorded in this study in Lublin. In turn, the pollen of this taxon accounts for a lower percentage in the aeroplankton in Southern Europe, e.g., 0.15% in Italy (Trieste) [46], 0.1% in Spain (Toledo) [47], and 0.6% in Greece (Thessaloniki) [48].

The global warming noted in the recent years has brought a number of changes in the course of plant flowering. More abundant pollen release has been recorded in the case of some tree species, e.g., *Quercus*, *Carya*, and *Betula* in the USA [49] and *Quercus* in Western Europe [50]. In the case of the willow pollen, we did not note either greater pollen abundance or earlier pollen season onsets. On the contrary, we recorded a downward trend in the pollen release abundance over the 16 years of the study. This trend has been noted in Lublin [51] and other study areas in Poland (Wrocław) as well as in the case of *Alnus* [52] and *Corylus* in Cracow [53].

A high value of pollen and nectar from various *Salix* species in terms of reward for insects was emphasized by Ostaff et al. [54] in Canada. The authors have found that *Salix* pollen and nectar may be highly important in early spring as a source of nutrition for pollinating insects. In early spring, pollinators play a prominent role as pollen vectors of crop plants (lowbush blueberry, cranberry, and apple). This group of insects includes Andrenidae, Apidae, Colletidae, Halictidae, and Syrphidae. In the surroundings of Lublin, we frequently observed representatives of Apidae, i.e., *Apis mellifera* and various species from the genus *Bombus*, visiting willow flowers.

Conclusions

The availability of *Salix* pollen to insects is closely associated with the onset of pollen seasons, which exhibits high variability in Lublin city, in SE Poland. The first *Salix* pollen grains appear in the air between the first 10-day-period of March and the second 10-day-period of April. The period of availability of the greatest amounts of *Salix* pollen in the area of Lublin lasts from the second 10-day-period of April to the first 10-day-period

of May. The variable airborne *Salix* pollen content in the subsequent years indicated a biennial cycle in the abundance of pollen release by the species of the analyzed genus, which is probably reflected in poorer supply of willow pollen reward every other year. In comparison with other parts of Poland, insects find better conditions in terms of *Salix* pollen supply in the area of Lublin. The aerobiological investigations demonstrate that the content of *Salix* pollen is higher in the urban area than 100 km away in the Roztoczański National Park.

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Ocena okresu dostępności pyłku *Salix* spp. dla owadów na podstawie badań aerobiologicznych

Streszczenie

Pyłek i nektar wytwarzany przez kwiaty gatunków z rodzaju *Salix* stanowią ważny pokarm w okresie wczesnowiosennym dla różnych grup owadów. Większość wierzb to gatunki owadopylne, jednakże w powietrzu rejestruje się znaczne ilości pyłku *Salix*. Celem badań było określenie zawartości pyłku tego taksonu w powietrzu Lublina (środkowo-wschodnia Polska) w latach 2001–2016 i wyznaczenie okresu jego największej dostępności dla owadów. W roku 2015 badaliśmy przebieg sezonów pyłkowych *Salix* w Lublinie oraz w Roztoczańskim Parku Narodowym. Stwierdziliśmy, że data początku sezonu pyłkowego znacznie się waha i przypada na okres zawarty między 16 marca a 17 kwietnia. Okres największej dostępności pyłku *Salix* dla owadów wyznaczono między końcem pierwszej dekady kwietnia a pierwszą dekadą maja. Średnia suma roczna ziaren pyłku *Salix*, zarejestrowanego w powietrzu, wyniosła 833. W Lublinie pyłek

Salix stanowi średnio 1.25% ogólnej zawartości pyłku różnych taksonów roślin w powietrzu. Z badań wynika, że istnieje dwuletni cykl obfitości pylenia *Salix*. Porównanie sezonów pyłkowych w Lublinie i Roztoczańskim Parku Narodowym wskazuje, że w powietrzu miasta występuje znacznie więcej pyłku niż w bioaerozolu Roztoczańskiego Parku Narodowego.